K113731

510(k) Summary

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Device Trade Name: **eSensor® Respiratory Viral Panel (RVP)**

Device Common Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay System

Measurand: Viral RNA/DNA of the following targets:

Target	Abrv.	Target	Abrv.
Influenza A	Flu A	Human Rhinovirus	HRV
Influenza A H1	Flu A H1	Human Metapneumovirus	hMPV
Influenza A H3	Flu A H3	Adenovirus B/E	ADV B/E
Influenza A 2009 H1N1	2009 H1N1	Adenovirus C	ADV C
Influenza B	Flu B	Parainfluenza Virus 1	PIV 1
Respiratory Syncytial Virus A	RSV A	Parainfluenza Virus 2	PIV 2
Respiratory Syncytial Virus B	RSV B	Parainfluenza Virus 3	PIV 3

Sample Type: Nasopharyngeal Swab (NPS)

Technology: Polymerase Chain Reaction (PCR)

Device Panel: OIVD Division of Immunology and Microbiology

Classification Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay, 866.3980
Instrumentation for clinical multiplex test systems, 862.2570

Classification Code: OCC: Respiratory viral panel multiplex nucleic acid assay
OEM: Human Metapneumovirus (hMPV) RNA assay system
OEP: Influenza A virus subtype differentiation nucleic acid assay
OOU: Parainfluenza Multiplex Nucleic Acid Assay
NSU: Instrumentation for clinical multiplex test systems

Predicate Device(s): Luminex® xTag™ RVP, 510(k) Number K081483
Classification Code: OCC, OEM, OEP
Regulation No. 866.3980
eSensor® Warfarin Sensitivity Test, 510(k) No. K073720
Classification Code (applicable): NSU Instrumentation for Clinical Multiplex Test Systems

Intended Use:

The eSensor® Respiratory Viral Panel (RVP) is a qualitative nucleic acid multiplex *in vitro* diagnostic test intended for use on the eSensor XT-8™ system for the simultaneous detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory infection.

The following virus types and subtypes are identified using the eSensor RVP: Influenza A, Influenza A H1 Seasonal Subtype, Influenza A H3 Seasonal Subtype, Influenza A 2009 H1N1 subtype, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Human Metapneumovirus, Human Rhinovirus, Adenovirus species B/E, and Adenovirus species C.

The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and epidemiological information.

Negative results do not preclude respiratory viral infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive results do not rule out bacterial infection, or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence and radiography) and clinical presentation must be taken into consideration in the final diagnosis of respiratory viral infection.

Performance characteristics for Influenza A were established during the 2010/2011 influenza season when Influenza A 2009 H1N1 and H3N2 were the predominant Influenza A viruses in circulation. When other Influenza A viruses emerge, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

For prescription use only.

Indication for Use: Same as Intended Use

Device Description:
The eSensor RVP is a multiplex microarray-based genotyping test system. It is based on the principles of competitive DNA hybridization using a sandwich assay format, wherein a single-stranded target binds concurrently to sequence-specific solution-phase signal probe and solid-phase electrode-bound capture probe. The test employs reverse transcription polymerase chain reaction amplification, exonuclease digestion and hybridization of target DNA/RNA. In the process, the double-stranded PCR amplicons are digested with exonuclease to generate single-stranded DNA suitable for hybridization. Hybridization occurs in the eSensor XT-8 Cartridge (described below) where the single-stranded target DNA is mixed with a hybridization solution containing labeled signal probes.

During hybridization, the single-stranded target DNA binds to a complementary, single-stranded capture probe immobilized on the working electrode surface. Single-stranded signal probes (labeled with electrochemically active ferrocenes) bind to the target adjacent to the capture probe. When inserted into the eSensor XT-8 instrument (described below), simultaneous hybridization of

target to signal probes and capture probe is detected by alternating current voltammetry (ACV). Each pair of working electrodes on the array contains a different capture probe, and sequential analysis of each electrode allows detection of multiple viral targets.

Table 1: Reagents supplied with the kit:

Box	Component	Packaging & Quantity	Storage
eSensor® Respiratory Viral Panel Cartridges	eSensor® RVP Cartridges	6 foil bags with 8 cartridges each in each cartridge pack	10-25 °C
	eSensor® Respiratory Viral Panel Product Insert	1 copy	Dry place (retain for reference)
eSensor® Respiratory Viral Panel Amplification Reagents	RVP Enzyme Mix	2 vials with 40 µL each	-15 to -30 °C (in a designated pre-PCR location)
	RVP PCR Mix	2 vials with 1000 µL each	
	MS2 Internal Control	2 vials with 300 µL each	
eSensor® Respiratory Viral Panel Detection Reagents	RVP Signal Buffer	2 vials with 2200 µL each	-15 to -30 °C (in a designated post-PCR location)
	Exonuclease	2 vials with 145 µL each .	
	Buffer-1	2 vials with 350 µL each	
	Buffer-2	2 vials with 700 µL each	

The Assay Cartridge (eSensor XT-8 Cartridge)

The eSensor XT-8 cartridge device consists of a printed circuit board (PCB) with a multi-layer laminate and a plastic cover that forms a hybridization chamber. The cartridge is fitted with a pump and check valves that circulate the hybridization solution when inserted into the eSensor XT-8 instrument. The PCB chip consists of an array of 72 gold-plated working electrodes, a silver reference electrode, and two gold-plated auxiliary electrodes. Each working electrode has a connector contact pad on the opposite side of the chip for electrical connection to the eSensor XT-8 instrument. Each electrode is modified with a multi-component; self-assembled monolayer that includes oligonucleotide capture probes specific for each polymorphic site on the test panel and insulator molecules. The cartridge also contains an electrically erasable programmable read-only memory component (EEPROM) that stores information related to the cartridge (e.g., assay identifier, cartridge lot number, and expiration date).

The eSensor XT-8 Instrument

The eSensor XT-8 instrument was previously cleared for IVD use by the FDA under K073720 and K090901.

The eSensor XT-8 is a clinical multiplex instrument that has a modular design consisting of a base module and one, two, or three cartridge-processing towers containing 8, 16, or 24 cartridge slots, respectively. The cartridge slots operate independently of each other. Any number of cartridges can be loaded at one time, and the remaining slots are available for use while the instrument is running.

The base module controls each processing tower, provides power, and stores and analyzes data. The instrument is designed to be operated solely with the touch screen interface. Entering patient accession numbers and reagent lot numbers can be performed by the bar code scanner or the touch screen.

Each processing tower consists of eight cartridge modules, each containing a cartridge connector, a precision-controlled heater, an air pump, and electronics. The air pumps drive the pump and valve system in the cartridge, eliminating fluid contact between the instrument and the cartridge. The pneumatic pumping enables recirculation of the hybridization solution allowing the target DNA and the signal probes to hybridize with the complementary capture probes on the electrodes. The pump in the cartridge is connected to a pneumatic source from the eSensor XT-8 instrument and provides unidirectional pumping of the hybridization mixture through the channel

during hybridization. Using this process to circulate the hybridization solution minimizes the unstirred boundary layer at the electrode surface and continuously replenishes the volume above the electrode that has been depleted of complementary targets and signal probes.

The XT-8 instrument provides electrochemical detection of bound signal probes by ACV and subsequent data analysis and test report generating functions. All hybridization, ACV scanning and analysis parameters are defined by a scanning protocol loaded into the XT-8 Software, and then specified for use by the EEPROM on each cartridge.

Principle of eSensor Technology: eSensor technology uses a solid-phase electrochemical method for determining the presence of one or more of a defined panel of virus target sequences. Purified DNA/RNA is isolated from the patient specimen according to defined laboratory procedures and the extracted nucleic acid is reverse transcribed and/or amplified using virus specific primers with an RT-PCR enzyme mix. The amplified DNA is converted to single-stranded DNA via exonuclease digestion and is then combined with a signal buffer containing ferrocene-labeled signal probes that are specific for the different viral targets. The mixture of amplified sample and signal buffer is loaded onto a cartridge containing single-stranded oligonucleotide capture probes bound to gold-plated electrodes. The cartridge is inserted into the XT-8 instrument where the single-stranded targets hybridize to the complementary sequences of the capture probes and signal probes, as shown in Figure 1. The presence of each target is determined by voltammetry, which generates specific electrical signals from the ferrocene-labeled signal probe.

The eSensor RVP provides a qualitative result based upon the presence (Positive) or absence (Target Not Detected) of the viruses contained in the panel along with the internal MS2 control. Positive and negative results are determined based on the electrical signals generated being either above or below specified signal boundaries, respectively.

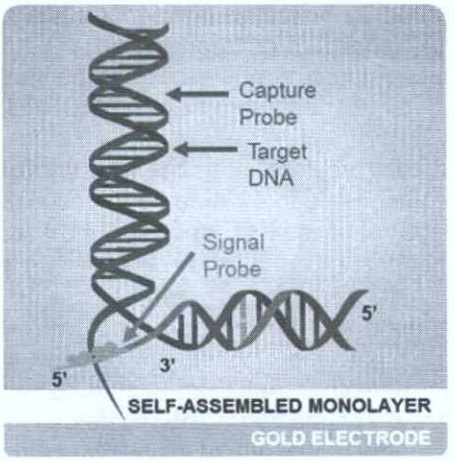

Figure 1: Hybridization complex formed at the surface of each electrode. Different, target specific, capture probes are covalently attached to the gold electrodes in the eSensor microarray. The amplified viral target DNA hybridizes to the capture probe and to a complementary ferrocene-labeled signal probe. The ferrocene label is detected at the electrode surface using voltammetry.

Substantial Equivalence Discussion: The eSensor Respiratory Viral Panel (RVP) uses the similar fundamental scientific technologies and has the same intended use as that of the predicate device, the Luminex® xTag® RVP and eSensor XT-8 Instrument. The eSensor XT-8 Instrument described in K078720 (eSensor Warfarin Sensitivity Test) is the identical instrument with a unique Assay Analysis Module (AAM) necessary to support the RVP IVD.

Table 2: Substantial Equivalence Predicate Comparison:

Element	GenMark eSensor RVP Subject Device	Luminex xTag RVP K081483
Intended Use	The eSensor® Respiratory Viral Panel (RVP) is a qualitative nucleic acid multiplex *in vitro* diagnostic test intended for use on the eSensor XT-8™ system for the simultaneous detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory infection. The following virus types and subtypes are identified using the eSensor RVP: Influenza A, Influenza A H1 Seasonal Subtype, Influenza A H3 Seasonal Subtype, Influenza A 2009 H1N1 subtype, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Human Metapneumovirus, Human Rhinovirus, Adenovirus species B/E, and Adenovirus species C. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and epidemiological information. Negative results do not preclude respiratory viral infection and should not be used as the	The xTAG RVP (Respiratory Viral Panel) is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in nasopharyngeal swabs from individuals suspected of respiratory tract infections. The following virus types and subtypes are identified using RVP: Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza 1, Parainfluenza 2, and Parainfluenza 3 virus, Human Metapneumovirus, Rhinovirus, and Adenovirus. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and laboratory findings. It is recommended that specimens found to be negative after examination using RVP be confirmed by cell culture. Negative results do not preclude respiratory viral infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Positive results do not rule out bacterial infection or co-infection with other organisms. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) and clinical presentation must be taken into consideration in order to obtain the final

Element	GenMark eSensor RVP Subject Device	Luminex xTag RVP K081483
	sole basis for diagnosis, treatment or other patient management decisions. Positive results do not rule out bacterial infection, or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence and radiography) and clinical presentation must be taken into consideration in the final diagnosis of respiratory viral infection. Performance characteristics for Influenza A were established during the 2010/2011 influenza season when Influenza A 2009 H1N1 and H3N2 were the predominant Influenza A viruses in circulation. When other Influenza A viruses emerge, performance characteristics may vary. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens. For prescription use only.	diagnosis of respiratory infection. Due to seasonal prevalence, performance characteristics for Influenza A/H1 were established primarily with retrospective specimens. The RVP assay cannot adequately detect Adenovirus species C, or serotypes 7a and 41. The RVP primers for detection of rhinovirus cross-react with enterovirus. A rhinovirus reactive result should be confirmed by an alternate method (e.g. cell culture). Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary. If infections with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to a state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Element	GenMark eSensor RVP Subject Device	Luminex xTag RVP K081483
Organisms Detected	Influenza A, Influenza A H1 Seasonal Subtype, Influenza A H3 Seasonal Subtype, Influenza A 2009 H1N1 strain, **Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3**, Human Metapneumovirus, Human Rhinovirus, **Adenovirus B/E and Adenovirus C**	Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza 1, Parainfluenza 2, and Parainfluenza 3 virus, Human Metapneumovirus, Rhinovirus, and Adenovirus
Specimen type	Same	Nasopharyngeal swabs (NPS)
Sample preparation	Same	Up front sample processing is required to extract nucleic acid
Assay technology	Same	PCR-based system for detecting viral nucleic acids in clinical specimens targeting unique regions of virus RNA/DNA
Detection technology	Solid phase electrochemical	Spectrofluorometry
Instrument	1. Same 2. eSensor XT-8	1. EasyMag® extraction system 2. Luminex® 100/200™ instrument
Software	Application software and embedded firmware (controls hardware functions) on XT-8 in addition to Assay Analysis Module (AAM) for RVP-IVD	IS or xPONENT software; xTAG Data Analysis Software RVP (US)
Time to results	Approximately 6 hours	Approximately 8 hours
Results	Same	Qualitative
Test interpretation	Automated test interpretation and report generation. User can access the raw signals generated by the assay.	Semi-automated test interpretation. User must review all "no call" results to determine cause and retesting strategy.
Controls	Same	Internal control added to each sample. External control processed with each batch of samples.

NON-CLINICAL PERFORMANCE DATA

Limit of Detection

Limit of Detection (LoD)

The Limit of Detection (LoD) was identified and verified for each viral target of the eSensor RVP using samples prepared from regrown and re-titered viral reference strains as detailed in Table 3. Initial estimations involved serially diluting each viral strain in M5 media. The lowest five dilutions prepared from each target were extracted in triplicate and the assay was performed once for each extract. Verification of the LoD was performed by meeting 95% detection or in at least 19 of the 20 extraction replicates on the selected dilution of each culture. Once the LoD was verified for each viral target in M5, verification of the LoD was also performed with the M4 media. Each viral target was diluted in M4 media down to the LoD concentration. The LoD dilution of each culture was extracted 20 times and the eSensor RVP assay was performed on each extract. The final results summary with the verified LoD concentration in $TCID_{50}$/mL for both M5 and M4 media is shown in Table 3.

Table 3: LoD Results Summary

Viral Target	Strain	Starting $TCID_{50}$/mL	LoD Spiked Conc. ($TCID_{50}$/mL)	% Positive
Flu A	H1N1 Brisbane/59/07	4.17×10^{5}	4.17×10^{-1}	100%
Flu A H1	H1N1 Brisbane/59/07	4.17×10^{5}	4.17×10^{-1}	97.5%
Flu A	H3N2	1.58×10^{8}	1.58×10^{3}	100%
Flu A H3	H3N2	1.58×10^{8}	1.58×10^{3}	100%
Flu A 2009 H1N1	NY/2009	1.05×10^{6}	1.05×10^{-1}	95%
Flu B	Florida/02/06	3.16×10^{6}	3.16×10^{-1}	100%
hMPV	B2	4.17×10^{5}	4.17×10^{0}	100%
HRV	3	1.58×10^{4}	1.58×10^{-3}	97.5%
PIV1	C35	2.81×10^{4}	2.81×10^{-2}	100%
PIV2	Greer	2.81×10^{6}	2.81×10^{0}	100%
PIV3	C 243	2.81×10^{7}	2.81×10^{1}	100%
RSV A	A2	2.81×10^{6}	2.81×10^{0}	97.5%
RSV B	9320	1.58×10^{5}	1.58×10^{0}	100%
ADV B/E	Type 4	1.58×10^{6}	1.58×10^{1}	100%
ADV C	Type 1	8.89×10^{7}	8.89×10^{1}	100%

Analytical Reactivity (Inclusivity)

Analytical Inclusivity Testing

The performance of the eSensor RVP with multiple viral target strains representing temporal and geographic diversity for each claimed viral target was evaluated. Each viral target strain was diluted in M5 transport media to a titer of 3X LoD for the corresponding viral target and extracted in triplicate using the bioMerieux NucliSENS easyMAG System. Following extraction, each

replicate was tested once using the eSensor RVP. In the case that a viral target strain is not detected at 3X LoD, 1000-fold serial dilutions were made from the viral stock and then each dilution was extracted in triplicate and tested using the eSensor RVP.

In cases where unexpected False Negative results were obtained, *in silico* analysis was performed. Table 4 shows the results.

Table 4: Analytical Reactivity (Inclusivity) Results

Target	Strain	Concentration Detected ($TCID_{50}$/ml)	LoD Multiple Detected
Flu A H1	A/New Caledonia/20/1999	4.2	10x
	A/Brisbane/59/07	1.26	3x
	FM/1/47H1	1.26	3x
	A/Denver/1/57	1.26	3x
	A/Solomon Islands/3/2006	1.26	3x
	A/Taiwan/42/06	1.26	3x
	A/NWS/33	1260	Flu A- 3x; H1- 3000x*
	A/PR/8/34	1.26	Flu A- 3x; H1- not detected*
	A/Mal/302/54	6372	Flu A- 3x H1- 15172x*
	A/Fort Monmouth/1/1947 (H1N1)	5.5	Flu A- 3x H1-13x*
Flu A H3	A/Aichi/2/68 H3N2	4743	3x
	A/Brisbane/10/07	4743	3x
	A/Victoria/3/75	4743	3x
	A/Port Chalmers/1/73	4743	3x
	A/Wisconsin/67/05	4743	3x
	A/Hong Kong/8/68	4743	3x
	A/Perth/16/2009	4743	3x
	Alice (vaccine) A/England/42/72	4743	3x
	MRC-2 Recombinant Strain	4743	3x
	A/Nanchang/933/95	4743	3x
Flu A 2009 H1N1	A/NY/02/2009	0.3	3x
	A/New Jersey/8/76	0.3	3x
	A/California/7/2009	0.3	3x

Target	Strain	Concentration Detected ($TCID_{50}$/ml)	LoD Multiple Detected
	A/Swine NY/01/2009	0.3	3x
	A/Swine NY/03/2009	0.3	3x
	A/Mexico/4108/09	0.3	3x
	A/Virginia/ATCC1/2009	0.3	3x
	A/Virginia/ATCC2/2009	0.6	Flu A- 3x 2009 H1N1- 6x**
	A/Virginia/ATCC3/2009	2.7	Flu A- 3x 2009 H1N1- 27x**
	A/Iowa/15/30	100	Flu A- 3x; 2009 H1N1- 1000x**
Flu B **(Victoria lineage except where noted)**	B/Florida/02/06 (Yamagata)	1	3x
	B/Malaysia/2506/04	1	3x
	B/Lee/40	1	3x
	B/Allen/45	1	3x
	B/GL/1739/54	1	3x
	B/Taiwan/2/62	1	3x
	B/Hong Kong/5/72	1	3x
	B/Maryland/1/59	1	3x
RSV A	A2	8.4	3x
	Long	8.4	3x
RSV B	9320	4.8	3x
	WV/14617/85	4.8	3x
	Wash/18537/62	4.8	3x
PIV1	C35	0.084	3x
	Type 1	0.084	3x
PIV2	Greer	8.4	3x
	Type 2	8.4	3x
PIV3	C-243	84	3x
	Type 3	84	3x
hMPV	IA3-2002 G, A1	12.6	3x
	IA14-2003 G, A2	12.6	3x
	Peru2-2002 G, B1	12.6	3x
	Peru6-2003 G, B2	12.6	3x

Target	Strain	Concentration Detected ($TCID_{50}$/ml)	LoD Multiple Detected
HRV A	1A	0.9	450x[#]
	A2	0.9	569x[#]
	A7	0.005	3x
	A16	0.005	3x
	18	Detected[##]	N/A
	A34	0.005	3x
	A57	0.005	3x
	A77	0.005	3x
	277G	0.2	100x[#]
HRV B	B3	0.1	80x
	B14	0.02	14x
	B17	0.4	253x
	B42	0.005	3x
	FO2-2547	0.2	89x[#]
	B83	0.2	127x
	84	Detected[##]	N/A
HRV C	C[$]	Detected[##]	N/A
ADV B	Type 3	0.3	3x
	Type 7A	0.3	3x
	Type 11 (lot 306523)	0.3	3x
	De Wit Type 14	0.3	3x
	Ch.79 Type 16	0.3	3x
	Type 21 (lot 307610)	0.3	3x
	Compton Type 34	0.3	3x
	Holden Type 35	0.3	3x
	Wan Type 50	0.3	3x
ADV C	Type 1	267	3x
	Type 2	533	6x
	Type 5	533	6x
	Type 6	533	6x
ADV E	Type 4	47	3x

* *In silico* analysis revealed little homology between the strain sequence and the H1 primer sequences. The degree of mis-match to the H1 subtype primer sequences resulted in decreased reactivity to these non-contemporary influenza strains for the H1 subtyping result.

** *In silico* analysis revealed little homology between the strain sequence and the 2009 H1N1 primer sequences. The degree of mis-match to the 2009 H1N1 subtype primer sequences resulted in decreased reactivity to these influenza strains for the 2009 H1N1 subtyping result.

HRV strain 3, used for LoD determination, had a $TCID_{50}$/ml of 0.0016. HRV strains 1A, FO2-2547, 277G were detected at a higher LoD multiple to the reference strain, respectively with their corresponding $TCID_{50}$/ml values of 0.9, 0.2, and 0.2. In silico analysis revealed mis-matches between the capture probe sequence and the HRV strains (2, 3 and 1 mis-match(es) respectively).

No concentration available since it was an extracted RNA sample.

$ Only one test done for HRV C due to limited sample availability

Supplemental Analytical Inclusivity Testing of Other Influenza Subtypes

Additional analytical inclusivity testing was carried out with either live isolates or purified genomic RNA of Influenza A strains.

Table 5: Additional Analytical Reactivity (Inclusivity) Results

Subtype	Host	Strain	Testing Conc.	RVP Result
Flu A H1N2	Human	A/NWS/34 (HA) x A/Rockefeller Institute/5/57 (NA), (H1N2), Reassortant NWS-F, RNA	0.74 ng	Flu A H1
Flu A H2N2	Avian	A/Japan/305/57, RNA	1.625 ng	Flu A
		A/Korea/426/68 (HA, NA) x A/Puerto Rico/8/34, RNA	3.12 ng	Flu A
Flu A H5N3		A/duck/Singapore/645/97, Wild Type	1.26 $TCID_{50}$/ml	Flu A
Flu A H10N7		A/chicken/Germany/N/49	1.26 $TCID_{50}$/ml	Flu A

Simulated Reactivity Information for Influenza Strains of Human, Swine, and Avian Origin

For any strains that were not available for testing with the eSensor RVP, such as Flu A H5 and Flu A H7 strains *in silico* analysis was performed. The eSensor RVP primers for Flu A, Flu A H1, Flu A 2009 H1N1 and Flu A H3 were aligned to the GenBank® sequences of the reactivity strains. Exclusivity was predicted based on the number and location of mismatches between assay primers and available strain sequences.

Simulated reactivity of the eSensor RVP with influenza strains was generated using a bioinformatics approach. Assay primer sequences, signal probes and capture probes were aligned with GenBank sequences corresponding to the appropriate gene targets and reactivity were predicated based on the number and location of mismatches in the targeted region shown in Table 6.

Table 6: Supplemental Reactivity of eSensor RVP Influenza A with Human, Swine, and Avian Influenza Strains

Subtype	Host	Strain	GenBank ID	Simulated RVP Reactivity Result
H2N2	Human	A/Albany/20/1957(H2N2)	CY022014	Flu A
	Avian	A/chicken/New York/13828-3/1995(H2N2)	CY014822	Flu A
		A/Japan/305/1957(H2N2)	CY014977	Flu A
		A/Korea/426/1968(H2N2)	CY031596	Flu A
H4N6	Avian	A/blue-winged teal/Minnesota/Sg-00043/2007(H4N6)	CY063978	Flu A
H5N1		A/peregrine falcon/Aomori/7/2011	AB629716	Flu A

Subtype	Host	Strain	GenBank ID	Simulated RVP Reactivity Result
		A/chicken/West Bengal/239022/2010	CY061305	Flu A
		A/chicken/West Bengal/193936/2009	GU272009	Flu A
		A/chicken/Hunan/1/2009	HM172150	Flu A
		A/chicken/Hunan/8/2008	GU182162	Flu A
		A/chicken/West Bengal/106181/2008	GU083632	Flu A
		A/chicken/Primorsky/85/2008	FJ654298	Flu A
	Avian	A/chicken/West Bengal/82613/2008	GU083648	Flu A
		A/duck/France/080036/2008	CY046185	Flu A
		A/duck/Vietnam/G12/2008	AB593450	Flu A
H5N1		A/chicken/Thailand/PC-340/2008	EU620664	Flu A
		A/great egret/Hong Kong/807/2008	CY036240	Flu A
		A/rook/Rostov-on-Don/26/2007(H5N1)	EU814504	Flu A
		A/turkey/VA/505477-18/2007(H5N1)	GU186510	Flu A
		A/chicken/Bangladesh/1151-10/2010(H5N1)	HQ156766	Flu A
	Human	A/Bangladesh/3233/2011	CY088772	Flu A
		A/Cambodia/R0405050/2007(H5N1)	HQ200572	Flu A
		A/Cambodia/S1211394/2008	HQ200597	Flu A
		A/Hong Kong/486/97(H5N1)	AF255368	Flu A
	Swine	A/swine/East Java/UT6010/2007(H5N1)	HM440124	Flu A
	Avian	A/duck/Pennsylvania/10218/1984(H5N2)	AB286120	Flu A
		A/American black duck/Illinois/08OS2688/2008	CY079453	Flu A
		A/American green-winged teal/California/HKWF609/2007	CY033447	Flu A
		A/Canada goose/New York/475813-2/2007	GQ923358	Flu A
		A/blue-winged teal/Saskatchewan/22542/2007	CY047705	Flu A
H5N2		A/chicken/Taiwan/A703-1/2008	AB507267	Flu A
		A/duck/France/080032/2008	CY046177	Flu A
		A/duck/New York/481172/2007	GQ117202	Flu A
		A/gadwall/Altai/1202/2007	CY049759	Flu A
		A/mallard/Louisiana/476670-4/2007	GQ923390	Flu A
		A/waterfowl/Colorado/476466-2/2007	GQ923374	Flu A
H5N3	Avian	A/duck/Singapore/F119/3/1997(H5N3)	GU052803	Flu A
H6N1		A/duck/PA/486/1969(H6N1)	EU743287	Flu A
H6N2		A/mallard/Czech Republic/15902-17K/2009(H6N2)	HQ244433	Flu A
H7N2	Avian	A/chicken/Hebei/1/2002	AY724263	Flu A
		A/chicken/PA/149092-1/02	AY241609	Flu A
		A/chicken/NJ/294508-12/2004	EU743254	Flu A
		A/chicken/New York/23165-6/2005	CY031077	Flu A
		A/muscovy duck/New York/23165-13/2005	CY033226	Flu A

Subtype	Host	Strain	GenBank ID	Simulated RVP Reactivity Result
		A/muscovy duck/New York/87493-3/2005	CY034791	Flu A
		A/mallard/Netherlands/29/2006	CY043833	Flu A
		A/northern shoveler/California/JN1447/2007	CY076873	Flu A
H7N2	Human	A/New York/107/2003(H7N2)	EU587373	Flu A
H7N3		A/Canada/rv504/2004(H7N3)	CY015007	Flu A
H7N7	Avian	A/American green-winged teal/Mississippi/09OS046/2009	CY079309	Flu A
		A/chicken/Germany/R28/03	AJ619676	Flu A
		A/chicken/Netherlands/1/03	AY340091	Flu A
		A/mallard/California/HKWF1971/2007	CY033383	Flu A
		A/mallard/Korea/GH171/2007	FJ959087	Flu A
		A/mute swan/Hungary/5973/2007	GQ240816	Flu A
		A/northern shoveler/Mississippi/ 09OS643/2009	CY079413	Flu A
	Human	A/Netherlands/219/03(H7N7)	AY340089	Flu A
H9N2		A/Hong Kong/1073/99(H9N2)	AJ278647	Flu A
	Avian	A/turkey/Wisconsin/1/1966(H9N2)	CY014664	Flu A
H11N9		A/duck/Memphis/546/1974(H11N9)	GQ257441	Flu A
H1N1	Swine	A/swine/Wisconsin/1/1971 (H1N1)	CY022414	Flu A
			CY022417	Flu A H1
	Human	A/California/UR06-0393/2007(H1N1)	CY026540	Flu A
			CY026539	Flu A H1
H1N2		A/New York/297/2003(H1N2)	CY002665	Flu A
			CY002664	Flu A H1
H1N1 (2009)		A/Aalborg/INS133/2009(H1N1)	CY063607	Flu A
			CY063606	2009 H1N1
H1N2	Swine	A/swine/Hong Kong/NS857/2001 (H1N2)	GQ229350	Flu A
			GQ229348	Flu A H1
		A/swine/Sweden/1021/2009(H1N2)	GQ495135	Flu A
			GQ495132	Flu A H1
H3N1	Avian	A/blue-winged teal/ALB/452/1983(H3N1)	CY004635	Flu A
			CY005940	Flu A H3
H3N2	Swine	A/swine/NY/A01104005/2011(H3N2)*	N/A*	Flu A
			JN940422	Flu A H3
		A/Maine/06/2011(H3N2)	JN866181	Flu A
			JN866186	Flu A H3
		A/Indiana/08/2011(H3N2)	JN655558	Flu A
			JN638733	Flu A H3
	Human	A/Boston/38/2008(H3N2)	CY044581	Flu A
			CY044580	Flu A H3

Subtype	Host	Strain	GenBank ID	Simulated RVP Reactivity Result
	Avian	A/American black duck/North Carolina/675-075/2004(H3N2)	GU051136	Flu A
			GU051135	Flu A H3
H3N5		A/mallard/Netherlands/2/1999(H3N5)	CY060264	Flu A
			CY060261	Flu A H3
H3N6		A/American black duck/New Brunswick/25182/2007(H3N6)	CY047697	Flu A
			CY047696	Flu A H3
H3N7		A/northern shoveler/California/HKWF1367/2007(H3N7)	CY033375	Flu A
			CY033372	Flu A H3
H3N8		A/American black duck/Washington/699/1978(H3N8)	GU052300	Flu A
			GU052299	Flu A H3

* Influenza A H3N2v (swine-origin). No sequence available but literature from CDC states that strain contains the conserved Matrix Protein (14) sequence from 2009 H1N1. Therefore Flu A should also be able to be detected.

Reproducibility

Multisite Reproducibility

Multisite reproducibility of the eSensor RVP was performed to evaluate the major sources of variability, such as lot-to-lot, extraction-to-extraction, site/instrument-to-site/instrument, day-to-day and operator/run-to-operator/run. Reproducibility testing occurred at three sites, utilizing a panel of samples containing viral material from culture derived isolates in media, simulating NPS specimen. Each of the selected panel targets was prepared at concentrations representing the following three viral load levels: Moderate Positive (100% positive, 3x LoD), Low Positive (95% positive, 1x LoD), and Negative (100% negative). Each simulated sample was divided into aliquots, blinded, and stored frozen (-70 °C) prior to testing. Each site received three lots of RVP Cartridges/Reagents, a set of samples for two operators and one XT-8 instrument. All samples were independently extracted using the bioMérieux easyMAG extraction system. Every analyte at each concentration was tested a total of 108 times (two operators at three sites, each testing three replicates on six testing days). Each lot of RVP Cartridges/Reagents was used in 36 tests per analyte/concentration. Summary results for each tested analyte are summarized below.

Table 7: Summary of Influenza A Calls (H3N2)

Flu A Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 1.3 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	243.0	23.6	9.7
	Site 2	36/36	0/36	100.0%	90.3%-100%	246.4	29.7	12.0
	Site 3	36/36	0/36	100.0%	90.3%-100%	235.0	32.6	13.9
	All Sites	108/108	0/108	100.0%	96.6%-100%	241.5	29.0	12.0
LOW POS (1x LoD) 0.4 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	248.3	28.6	11.5
	Site 2	36/36	0/36	100.0%	90.3%-100%	244.7	26.4	10.8
	Site 3	36/36	0/36	100.0%	90.3%-100%	232.8	23.2	10.0
	All Sites	108/108	0/108	100.0%	96.6%-100%	242.0	26.7	11.1

Flu A Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
Negative	Site 1	3/288	285/288	99.0%	97.0%-99.8%	1.2	2.4	N/A
	Site 2	1/288	287/288	99.7%	98.1%-100%	1.0	2.2	N/A
	Site 3	2/288	286/288	99.3%	97.5%-99.9%	1.0	0.9	N/A
	All Sites	6/864	858/864	99.3%	98.5%-99.7%	1.1	1.9	N/A

Table 8: Summary of Influenza A H3 Calls

Flu A H3 Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 4.7×10^3 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	86.5	23.9	27.7
	Site 2	36/36	0/36	100.0%	90.3%-100%	77.4	26.7	34.5
	Site 3	36/36	0/36	100.0%	90.3%-100%	86.0	30.5	35.4
	All Sites	108/108	0/108	100.0%	96.6%-100%	83.3	27.3	32.7
LOW POS (1x LoD) 1.6×10^3 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	81.5	26.1	32.0
	Site 2	36/36	0/36	100.0%	90.3%-100%	68.2	29.6	43.4
	Site 3	36/36	0/36	100.0%	90.3%-100%	84.9	21.3	25.1
	All Sites	108/108	0/108	100.0%	96.6%-100%	78.2	26.6	34.1
Negative	Site 1	0/288	288/288	100.0%	98.7%-100%	0.4	0.3	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	0.3	0.3	N/A
	Site 3	1/288	287/288	99.7%	98.1%-100%	0.4	0.4	N/A
	All Sites	1/864	863/864	99.9%	99.4%-100%	0.4	0.4	N/A

Table 9: Summary of Adenovirus B/E Calls

ADV B Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 47.4 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	109.1	10.9	10.0
	Site 2	36/36	0/36	100.0%	90.3%-100%	102.6	11.5	11.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	102.2	14.5	14.1
	All Sites	108/108	0/108	100.0%	96.6%-100%	104.7	12.7	12.1
LOW POS (1x LoD) 15.8 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	92.7	11.4	12.3
	Site 2	36/36	0/36	100.0%	90.3%-100%	89.9	10.9	12.1
	Site 3	36/36	0/36	100.0%	90.3%-100%	84.5	16.9	20.0
	All Sites	108/108	0/108	100.0%	96.6%-100%	89.1	13.7	15.3
Negative	Site 1	1/288	287/288	99.7%	98.1%-100%	1.6	6.0	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	1.2	0.4	N/A
	Site 3	0/288	288/288	100.0%	98.7%-100%	1.2	0.4	N/A
	All Sites	1/864	863/864	99.9%	99.4%-100%	1.3	3.5	N/A

Table 10: Summary of hMPV Calls

hMPV Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 13 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	91.2	26.1	28.6
	Site 2	36/36	0/36	100.0%	90.3%-100%	92.5	37.1	40.1
	Site 3	36/36	0/36	100.0%	90.3%-100%	100.4	22.7	22.6
	All Sites	108/108	0/108	100.0%	96.6%-100%	94.7	29.3	30.9
LOW POS (1x LoD) 4 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	56.4	30.0	53.2
	Site 2	35/36	1/36	97.2%	85.5%-99.9%	51.0	31.2	61.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	63.8	28.1	44.0
	All Sites	107/108	1/108	99.1%	94.9%-100%	57.1	30.0	52.5
Negative	Site 1	0/288	288/288	100.0%	98.7%-100%	0.1	0.0	N/A
	Site 2	8/288	280/288	97.2%	94.6%-98.8%	0.7	4.1	N/A
	Site 3	0/288	288/288	100.0%	98.7%-100%	0.1	0.1	N/A
	All Sites	8/864	856/864	99.1%	98.2%-99.6%	0.3	2.4	N/A

Table 11: Summary of PIV 3 Calls

PIV3 Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 84 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	178.1	27.4	15.4
	Site 2	36/36	0/36	100.0%	90.3%-100%	193.8	29.8	15.4
	Site 3	36/36	0/36	100.0%	90.3%-100%	160.8	27.4	17.0
	All Sites	108/108	0/108	100.0%	96.6%-100%	177.6	31.1	17.5
LOW POS (1x LoD) 28 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	139.0	34.8	25.1
	Site 2	36/36	0/36	100.0%	90.3%-100%	162.4	27.9	17.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	126.9	38.9	30.7
	All Sites	108/108	0/108	100.0%	96.6%-100%	142.8	37.0	25.9
Negative	Site 1	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A
	Site 3	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A
	All Sites	0/864	864/864	100.0%	99.6%-100%	0.2	0.1	N/A

Table 12: Summary of RSV A Calls

RSV A Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 8.4 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	166.3	19.2	11.5
	Site 2	36/36	0/36	100.0%	90.3%-100%	156.7	31.7	20.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	156.4	22.9	14.7
	All Sites	108/108	0/108	100.0%	96.6%-100%	159.8	25.3	15.8
LOW POS (1x LoD) 2.8 $TCID_{50}$/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	146.6	22.7	15.5
	Site 2	36/36	0/36	100.0%	90.3%-100%	124.6	41.0	32.9
	Site 3	35/36	1/36	97.2%	85.5%-99.9%	128.2	33.4	26.1
	All Sites	107/108	1/108	99.1%	94.9%-100%	133.1	34.3	25.8
Negative	Site 1	4/288	284/288	98.6%	96.5%-99.6%	0.7	4.0	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A

RSV A Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
	Site 3	0/288	288/288	100.0%	98.7%-100%	0.2	0.2	N/A
	All Sites	4/864	860/864	99.5%	98.8%-99.9%	0.4	2.3	N/A

Testing of Dual Infection Samples

Clinically Relevant Co-Infections

An internal co-infection study was performed to determine the capability of the eSensor RVP to detect clinically relevant dual co-infections in patient samples. Nine clinically relevant co-infections were evaluated in this study. Dual co-infections were prepared by using the representative viral cultures at two different concentrations - Virus A at 1x LoD and Virus B at 10,000x LoD, as well as Virus A at 10,000x LoD and Virus B at 1x LoD. Relevant medical literature was sourced for selection of viral mix composition of common or expected co-infections.

The table below summarizes the $TCID_{50}$/ml and LoD multiple detected in each viral co-infection.

Table 13: Dual Infection Reproducibility Summary Results

Viral Co-Infection (Virus 1/Virus 2)	Virus 1 Detected		Virus 2 Detected	
	LoD Multiple	$TCID_{50}$/ml	LoD Multiple	$TCID_{50}$/ml
H3-RSV	1x LoD	1.58×10^{3}	10,000x LoD	2.81×10^{4}
RSV-H3	3x LoD	8.43×10^{0}	10,000x LoD	1.58×10^{7}
H3-FLUB	1x LoD	1.58×10^{3}	10,000x LoD	3.16×10^{3}
FLUB-H3	1x LoD	3.16×10^{-1}	10,000x LoD	1.58×10^{7}
H1N1-HRV	3x LoD	3.15×10^{-1}	10,000x LoD	1.58×10^{1}
HRV-H1N1	1x LoD	1.58×10^{-3}	10,000x LoD	1.05×10^{3}
H1N1-PIV3	1x LoD	1.05×10^{-1}	10,000x LoD	2.81×10^{5}
PIV3-H1N1	1x LoD	2.81×10^{1}	10,000x LoD	1.05×10^{3}
H1N1-RSV	1x LoD	1.05×10^{-1}	10,000x LoD	2.81×10^{4}
RSV-H1N1	3x LoD	8.43×10^{0}	10,000x LoD	1.05×10^{3}
RSV-ADV	1x LoD	2.81×10^{0}	10,000x LoD	8.89×10^{2}
ADV-RSV	1x LoD	8.89×10^{-2}	10,000x LoD	2.81×10^{4}
HMPV-RSV	1x LoD	4.17×10^{0}	10,000x LoD	2.81×10^{4}
RSV-HMPV	1x LoD	2.81×10^{0}	10,000x LoD	4.17×10^{4}
HMPV-ADV	1x LoD	4.17×10^{0}	10,000x LoD	8.89×10^{2}
ADV-HMPV	1x LoD	8.89×10^{-2}	10,000x LoD	4.17×10^{4}
HRV-RSV	3x LoD	4.74×10^{-3}	10,000x LoD	2.81×10^{4}
RSV-HRV	1x LoD	2.81×10^{0}	10,000x LoD	1.58×10^{1}

Interference

Interfering Substances

Potentially interfering substances were selected based on the fact that they could pre-exist in the specimen (e.g. blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms) as well as those that could be introduced during specimen collection and preparation. Each potentially interfering substance was tested individually with the exception of Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum, and Sulfur, which were tested together as Zicam® Allergy Relief Nasal spray and Oxymetazoline and Menthol, which were tested together as Afrin® No Drip Severe Congestion nasal spray, thereby bringing the total to 21 potentially interfering test combinations. Viral samples representative of the 14 viral targets on the eSensor RVP were obtained from commercially available cultured cell lines as indicated in Table 14. Seven viral mixes were made, each containing unique viral targets. Viral mixes were added to each potentially interfering substance resulting in a final testing concentration of 3X LoD for each analyte. Each was extracted in triplicate with each extract tested once with the eSensor RVP. Twenty-four (24) potentially interfering substances were tested in this study with 21 combinations. Additionally, nine potentially interfering microorganisms (viral and bacterial) were also tested in the same manner as described above. The microorganisms and their testing concentrations are listed in Table 14. All substances and microorganisms tested for interference were shown to be compatible with the eSensor RVP. No potentially interfering substance or microorganism was shown to inhibit the eSensor RVP at all tested concentrations[a].

Table 14: Potentially Interfering Substances

Potentially Interfering Substance	Active Ingredient	Substance Form	Tested Concentration
Sample Matrix	Control for no interfering substance	Liquid	N/A
Viral transport medium	Becton Dickinson VTM	Liquid	N/A
Blood (human)	Blood	Liquid	2% v/v
	Human gDNA	50 ng/rxn	50 ng/rxn
Throat lozenges, oral anesthetic and analgesic	Benzocaine	Dry	30% w/v
	Menthol*	Nasal Spray	1% v/v
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein	Dry	1% w/v
Nasal sprays or drops	Phenylephrine (Neo-Synephrine)	Dry	1.5% v/v
	Oxymetazoline* (also contains Benzalkonium Chloride, Menthol, Eucalyptol, Camphor, benzyl alcohol and phosphate buffers)	Nasal Spray	1% v/v
	Sodium chloride	Dry	0.8% w/v
Antibacterial, systemic	Tobramycin	Dry	5% w/v
Antibiotic, nasal ointment	Mupirocin	Dry	2% w/v
Nasal corticosteroids	Beclomethasone	Dry	1.5% w/v
	Dexamethasone	Dry	1.5% w/v
	Flunisolide	Dry	1.5% w/v
	Triamcinolone	Dry	1.5% w/v
	Budesonide (Pulmicort)	Dry	1.5% w/v
	Fluticasone (Flonase©)	Dry	3% w/v
Nasal gel	Luffa opperculata**	Nasal Gel	1% v/v
	Sulfur**	Nasal Gel	1% v/v

Potentially Interfering Substance	Active Ingredient	Substance Form	Tested Concentration
Homeopathic allergy relief medicine	Galphimia glauca**	Nasal Gel	1% v/v
	Histaminum hydrochloricum**	Nasal Gel	1% v/v
FluMist®#	Live intranasal influenza virus vaccine#	Liquid	0.5%-1% v/v
Anti-viral drugs	Zanamivir (Relenza®)	Dry	550 ng/ml
	Oseltamivir (Tamiflu®)	Dry	142 ng/ml
Virus	Cytomegalovirus	Culture	1 x 10^5 PFU/mL
	Enterovirus 71	Culture	
Bacteria	Streptococcus pneumoniae	Culture	1 x 10^6 CFU/mL
	Bordetella pertussis	Culture	
	Haemophilus influenza	Culture	
	Mycoplasma pneumoniae	Culture	
	Staphylococcus aureus	Culture	
	Neisseria meningitidis	Culture	
	Corynebacterium diptheriae	Culture	

*Tested together (Afrin No Drip Severe Congestion nasal spray)
** Tested together (Zicam Allergy Relief)
# FluMist vaccine: Addition of FluMist Live Intranasal Influenza Vaccine to the transport media control resulted in positive calls for Flu A, Flu A H3, Flu A 2009 H1N1 and Flu B. This was due to the live attenuated influenza virus present in the vaccine.
[a] Testing of FluMist at 1% (v/v) resulting in an inhibition in the detection of hMPV. FluMist did not inhibit the detection of hMPV when tested at 0.5% (v/v).

Cross-Reactivity

Cross-Reactivity Evaluation for Viruses Detected by the eSensor RVP

Cross-reactivity of each viral target (14 viral targets) was evaluated at high concentrations with the eSensor RVP by making three serial dilutions of viral reference strains with viral transport media (Remel M5) at 10,000x, 1000x and 100x the LoD. The titer of each virus dilution and corresponding LoD values were determined and provided in the table below. Cross-reactivity was not observed with any of the RVP viral targets at the concentrations tested. Table 15 summarizes the cross-reactivity results.

Table 15: Within Panel Cross-Reactivity Final Results

Viral Target	Strain	LoD Concentration ($TCID_{50}$/mL)	Highest Test Concentration ($TCID_{50}$/mL)	Highest Multiple of LoD Tested	Cross-Reactivity Results
Flu A	H1N1 Brisbane/59/07	4.17 x 10^{-1}	4.17 x 10^3	10,000x	Not Observed
Flu A H1	H1N1 Brisbane/59/07	4.17 x 10^{-1}	4.17 x 10^3	10,000x	Not Observed
Flu A	H3N2	1.58 x 10^3	1.58 x 10^7	10,000x	Not Observed
Flu A H3	H3N2	1.58 x 10^3	1.58 x 10^7	10,000x	Not Observed
Flu A 2009 H1N1	NY/2009	1.05 x 10^{-1}	1.05 x 10^3	10,000x	Not Observed
Flu B	Florida/02/06	3.16 x 10^{-1}	3.16 x 10^3	10,000x	Not Observed
hMPV	B2	4.17 x 10^0	4.17 x 10^4	10,000x	Not Observed
HRV	3	1.58 x 10^{-3}	1.58 x 10^1	10,000x	Not Observed
PIV1	C35	2.81 x 10^{-2}	2.81 x 10^2	10,000x	Not Observed

Viral Target	Strain	LoD Concentration ($TCID_{50}$/mL)	Highest Test Concentration ($TCID_{50}$/mL)	Highest Multiple of LoD Tested	Cross-Reactivity Results
PIV2	Greer	2.81×10^{0}	2.81×10^{4}	10,000x	Not Observed
PIV3	C 243	2.81×10^{1}	2.81×10^{5}	10,000x	Not Observed
RSV A	A2	2.81×10^{0}	2.81×10^{4}	10,000x	Not Observed
RSV B	9320	1.58×10^{0}	1.58×10^{4}	10,000x	Not Observed
ADV B/E	Type 7 Type 4	8.89×10^{-2} 1.58×10^{1}	1.58×10^{5}	10,000x	Not Observed
ADV C	Type 1	8.89×10^{1}	8.89×10^{5}	10,000x	Not Observed

Cross-Reactivity with Other Respiratory Viruses Not Targeted by the eSensor RVP
Cross-reactivity with 5 respiratory viruses known to circulate with low frequency in the general population was assessed. All viral strains were diluted in M5 transport media to a titer of 10^5 PFU/mL and extracted using the bioMérieux easyMAG extraction method in triplicate. Following extraction, each replicate was tested once in the RVP assay.

Table 16: Cross-Reactivity Results of Rare Respiratory Virus

Organism	Source	Test Concentrations	Cross-Reactivity Results
Parainfluenza Virus 4	Zeptometrix	2.92×10^{5} PFU/mL	Not Observed
Coronavirus OC43*	Zeptometrix	5.96×10^{4} PFU/mL	Not Observed
Coronavirus 229E	Zeptometrix	1.36×10^{5} PFU/mL	Not Observed
Coronavirus NL63**	Zeptometrix	9.89×10^{4} PFU/mL	Not Observed
Coronavirus HKU1	Clinical Isolate	N/A[$]	Not Observed

**OC43 had one replicate fail the IC control at high (10^5) concentration.*
***NL63 was tested at the highest concentration available - 9.89×10^4 PFU/mL.*
[$]The Coronavirus HKU1 sample was a clinical isolate identified during the method comparison study. The method used was qualitative so no copy information was available.

Cross-Reactivity with 17 additional viruses that are not targets of the eSensor RVP were also assessed (Table 17.) All viral strains were diluted in M5 transport media to a titer of 10^5 PFU/mL and extracted using the bioMérieux easyMAG extraction method in triplicate reactions.

Table 17: Cross- Reactivity Results of with other Viruses

Organism	Source	Test Concentrations	Cross-Reactivity Results
Adenovirus 18 (A)	Zeptometrix VPL-030	2.37×10^{5} PFU/mL	Not Observed
Adenovirus 9 (D)	Zeptometrix VPL-030	4.63×10^{5} PFU/mL	ADV C False Positive*
Adenovirus 41 (F)	Zeptometrix VPL-030	8.05×10^{5} PFU/mL	ADV C False Positive*
Enterovirus 71	Zeptometrix 0810047CF	2.92×10^{5} PFU/mL	Not Observed
Coxsackievirus A10	Zeptometrix 0810106CF	1.72×10^{5} PFU/mL	Not Observed
Coxsackievirus A9	Zeptometrix 0810017CF	2.21×10^{5} PFU/mL	Not Observed
Echovirus E6	Zeptometrix 0810076CF	7.16×10^{5} PFU/mL	Not Observed
Coxsackievirus B2	ATCC VR-29	6.22×10^{6} PFU/mL	Not Observed
Coxsackievirus B3	Zeptometrix 0810074CF	1.06×10^{5} PFU/mL	Not Observed
Coxsackievirus B4	Zeptometrix 0810075CF	8.04×10^{6} PFU/mL	2/3 Not Observed 1 HRV Positive**

Organism	Source	Test Concentrations	Cross-Reactivity Results
Coxsackievirus B5	Zeptometrix 081019CF	7.16 x 10^7 PFU/mL	Not Observed
Echovirus 9	Zeptometrix 081007CF	1.41 x 10^5 PFU/mL	Not Observed
Echovirus 25	Zeptometrix VPL-030	1.93 x 10^5 PFU/mL	Not Observed
Echovirus 30	Zeptometrix 0810078CF	9.89 x 10^4 PFU/mL	Not Observed
Coxsackievirus A21	Zeptometrix 0810018CF	2.92 x 10^5 PFU/mL	Not Observed
Coxsackievirus A24	ATCC VR-583	7.00 x 10^5 PFU/mL	Not Observed
Enterovirus 68	ATCC VR-561	1.40 x 10^5 PFU/mL	Not Observed
Poliovirus	ATCC VR-193	1.11 x 10^5 PFU/mL,	HRV False Positive[$]
Bocavirus	Clinical Isolate	N/A	Not Observed
Herpesvirus 1: Herpes Simplex	Zeptometrix 0810005CF	1.01 x 10^5 PFU/mL	Not Observed
Herpesvirus 3: Varicella Zoster	Zeptometrix 081 0026CF	2.35 x 10^6 copies/mL[#]	Not Observed
Herpesvirus 4: Epstein Barr	Zeptometrix 0810008CF	1.06 x 10^5 PFU/mL	Not Observed
Herpesvirus 5: Cytomegalovirus	Zeptometrix 0810003CF	6.68 x 10^5 PFU/mL	Not Observed
Measles	Zeptometrix	1.37 x 10^5 PFU/mL	Not Observed
Mumps	Zeptometrix 0810079CF	1.93 x 10^5 PFU/mL	Not Observed

*ADV C cross-reactive signal was also obtained from Adenovirus 9 (D) and Adenovirus 41 (F) when it was diluted 1000 fold from the initial testing concentration. Due to the genetic similarity between Adenovirus C, D, and F, the eSensor RVP cannot reliably differentiate them. A positive eSensor RVP Adenovirus species C result should be followed-up using an alternative method (e.g., sequence analysis) if definitive Adenovirus speciation is needed.

One replicate of Coxsackievirus B4 at high concentration (8.04 x 10^6 PFU/mL) generated a HRV positive result which was slightly above the assay threshold. None of the other thirteen (13) enterovirus analytes tested at similar high concentrations generated a positive call for HRV.

[$]Due to the genetic similarity between human rhinovirus and poliovirus, the eSensor RVP cannot reliably differentiate them. If a polio infection is suspected, a positive eSensor RVP human rhinovirus (HRV) result should be confirmed using an alternate method (e.g., cell culture).

[#] Quantification of the viral RNA contained in the Herpesvirus-3 (Varicella Zoster Virus) sample was performed using real-time RT-PCR and provided in copies/mL

Cross-Reactivity with Bacteria and Fungus

Bacterial and fungal strains were tested for cross-reactivity with the eSensor RVP and were diluted in M5 transport media to a titer of 10^6 CFU/mL. These organisms were extracted in triplicate with the bioMérieux easyMAG system.

Following extraction, each replicate was tested once using the eSensor RVP as shown in Table 18.

Table 18: Cross-Reactivity Results of with Bacteria and Fungus

Organism	Source	Test Concentrations	Cross-Reactivity Results
Acinetobacter baumanii	Zeptometrix 0801597	5.2 x 10^6 CFU/mL	Not Observed
Bordetella parapertussis	Zeptometrix 0801461	9.8 x 10^6 CFU/mL	Not Observed
Bordetella pertussis	Zeptometrix 0801459	5.8 x 10^6 CFU/mL	Not Observed
Burkholderia cepacia	Zeptometrix BacT-050	2.3 x 10^6 CFU/mL	Not Observed
Candida albicans	Zeptometrix 0801504	1.0 x 10^6 CFU/mL	Not Observed
Candida glabrata	Zeptometrix 0801535	9.73 x 10^6 CFU/mL	Not Observed
Chlamydophila pneumoniae DNA	ABI 08-942-250	1.4 x 10^7 copies/mL	Not Observed
Corynebacterium diphtheriae	Zeptometrix BacT-050	3.58 x 10^6 CFU/mL	Not Observed

Organism	Source	Test Concentrations	Cross-Reactivity Results
Escherichia coli	Zeptometrix 0801624	1.5 x 10^6 CFU/mL	Not Observed
Haemophilus influenzae	Zeptometrix 0801680	2.6 x 10^6 CFU/mL	Not Observed
Klebsiella pneumoniae	Zeptometrix 0801506	1.07 x 10^6 CFU/mL	Not Observed
Lactobacillus acidophilus	Zeptometrix 0801540	2.12 x 10^6 CFU/mL	Not Observed
Lactobacillus planarum	Zeptometrix 0801507	1.75 x 10^6 CFU/mL	Not Observed
Legionella pneumophila	Zeptometrix 0801645	2.6 x 10^6 CFU/mL	Not Observed
Moraxella catarrhalis	Zeptometrix 0801509	3.9 x 10^6 CFU/mL	Not Observed
Mycobacterium tuberculosis	Zeptometrix 0801660	2.2 x 10^6 CFU/mL	Not Observed
Mycoplasma pneumoniae	Zeptometrix 0801579	2.47 x 10^6 CCU/mL	Not Observed
Neisseria meningiditis	Zeptometrix 0801511	3.37 x 10^6 CFU/mL	Not Observed
Neisseria sicca	Zeptometrix 0801754	3.37 x 10^6 CFU/mL	Not Observed
Porphyromonas gingivalis	Zeptometrix BacT-050	3.55x10^6 CFU/mL	Not Observed
Proteus vulgaris	Zeptometrix BacT-050	1.0 x 10^6 CFU/mL	Not Observed
Pseudomonas aeruginosa	Zeptometrix 0801519	1.05 x 10^6 CFU/mL	Not Observed
Serratia marcescens	Zeptometrix 0801723	6.1 x 10^6 CFU/mL	Not Observed
Staphylococcus aureus (COL)	Zeptometrix 0801638	8.4 x 10^6 CFU/mL	Not Observed
Staphylococcus aureus (MSSA)	Zeptometrix 0801675	1.2 x 10^6 CFU/mL	Not Observed
Staphylococcus epidermidis (MSSE)	Zeptometrix 0801689	2.2 x 10^6 CFU/mL	Not Observed
Staphylococcus epidermidis (MRSE)	Zeptometrix 0801651	6.2 x 10^6 CFU/mL	Not Observed
Staphylococcus haemolyticus	Zeptometrix 0801591	2.16 x 10^6 CFU/mL	Not Observed
Streptococcus agalactiae	Zeptometrix 0801545	2.2 x 10^6 CFU/mL	Not Observed
Streptococcus dysgalactiae	Zeptometrix 0801516	6.46 x 10^6 CFU/mL	Not Observed
Streptococcus mitis	Zeptometrix 0801695	2.43 x 10^6 CFU/mL	Not Observed
Streptococcus pneumoniae	Zeptometrix 0801439	2.8 x 10^6 CFU/mL	Not Observed
Streptococcus pyrogenes	Zeptometrix 0801512	1.55 x 10^6 CFU/mL	Not Observed
Streptococcus salivarius	Zeptometrix BacT-050	6.53 x 10^6 CFU/mL	Not Observed

Carryover/Cross-Contamination

The carryover/cross-contamination study challenged the extraction, RT-PCR, and detection portions of the assay within and between runs and operators tested over the course of five testing days. A representative strain of Parainfluenza Virus 3 was obtained as a commercially available cultured cell line. Positive Parainfluenza Virus 3 samples were prepared at a concentration of 1.00 x 10^5 $TCID_{50}$/mL (3559x LoD) while negative samples were un-inoculated Remel M5 transport media. All samples were extracted using the bioMérieux easyMAG System. Five sets of alternating high concentration positive and negative samples were extracted and tested in a checkerboard pattern. Each set of samples contained 24 tests (12 positive and 12 negative). Total number of tests for the duration of the study was 120 samples (60 positive and 60 negative).

No carryover/cross-contamination was observed in the eSensor RVP, as 100% of the PIV 3 negative samples were reported as 'Target Not Detected'.

CLINICAL PERFORMANCE DATA

Clinical Performance

Expected Values

A prospective clinical study testing nasopharyngeal (NP) swab specimens was conducted during the 2010/11 influenza season at three North American clinical laboratories. The expected values of individual analytes based on eSensor RVP results in prospective samples are summarized in Tables 19 and 20. The expected values of mixed co-infections based on eSensor RVP results in prospective samples are summarized in Tables 21 and 22.

Table 19: Expected Value (As Determined by eSensor RVP) Summary by Age Group in the Prospective Clinical Evaluation

Virus (Analyte)	Age 0-1 (N = 270)	Age >1-5 (N = 136)	Age >5-21 (N = 127)	Age >21-65 (N = 333)	Age >65 (N = 171)	All Ages (N = 1037)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Influenza A (Un-Subtypable)	2 (0.7)	0 (0.0)	2 (1.6)	5 (1.5)	1 (0.6)	**10 (1.0)**
Influenza A (Total)	25 (9.3)	22 (16.2)	17 (13.4)	84 (25.2)	31 (18.1)	**179 (17.3)**
Influenza A H3	12 (4.8)	15 (11.0)	7 (5.5)	43 (12.9)	22 (12.9)	**99 (9.5)**
Influenza A 2009 H1N1	10 (3.7)	8 (5.9)	6 (4.7)	33 (9.9)	7 (4.1)	**64 (6.2)**
Influenza B	10 (3.7)	17 (12.5)	33 (26.0)	15 (4.5)	7 (4.1)	**82 (7.9)**
Human Metapneumovirus	18 (6.7)	11 (8.1)	3 (2.4)	15 (4.5)	10 (5.9)	**57 (5.5)**
Human Rhinovirus	82 (30.4)	27 (19.9)	21 (16.6)	26 (7.8)	11 (6.4)	**167 (16.1)**
Parainfluenza Virus 1	3 (1.1)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.6)	**5 (0.5)**
Parainfluenza Virus 2	1 (0.4)	1 (0.7)	0 (0.0)	3 (0.9)	2 (1.2)	**7 (0.7)**
Parainfluenza Virus 3	43 (15.9)	15 (11.0)	5 (3.9)	18 (5.4)	5 (2.9)	**86 (8.3)**
Respiratory Syncytial Virus A	69 (25.6)	21 (15.4)	8 (6.3)	11(3.3)	10 (5.8)	**119 (11.4)**
Respiratory Syncytial Virus B	28 (10.4)	17 (12.5)	4 (3.2)	14 (4.2)	6 (3.5)	**69 (6.7)**
Adenovirus B/E	6 (2.2)	8 (5.9)	3 (1.6)	5 (1.8)	0 (0.0)	**22 (2.1)**
Adenovirus C	21 (7.7)	4 (2.9)	1 (0.8)	9 (2.7)	6 (3.5)	**41 (3.9)**

Table 20: Expected Value (As Determined by eSensor RVP) Summary by Site in the Prospective Clinical Evaluation

Virus (Analyte)	Site 1 (N = 245)	Site 2 (N = 533)	Site 3 (N = 259)	All Sites (N = 1037)
	N (%)	N (%)	N (%)	N (%)
Influenza A (Un-Subtypable)	0 (0.0)	8 (1.5)	2 (0.8)	**10 (1.0)**
Influenza A (Total)	58 (23.7)	89 (16.7)	32 (12.4)	**179 (17.3)**
Influenza A H3	32 (13.1)	54 (10.1)	13 (5.0)	**99 (9.5)**

Virus (Analyte)	Site 1 (N = 245)	Site 2 (N = 533)	Site 3 (N = 259)	All Sites (N = 1037)
	N (%)	N (%)	N (%)	N (%)
Influenza A 2009 H1N1	19 (7.8)	28 (5.3)	17 (6.6)	**64 (6.2)**
Influenza B	4 (1.6)	59 (11.1)	19 (7.3)	**82 (7.9)**
Human Metapneumovirus	23 (9.4)	25 (4.7)	9 (3.5)	**57 (5.5)**
Human Rhinovirus	44 (18.0)	99 (18.6)	24 (9.3)	**167 (16.1)**
Parainfluenza Virus 1	0 (0.0)	4 (0.8)	1 (0.4)	**5 (0.5)**
Parainfluenza Virus 2	1 (0.4)	6 (1.1)	0 (0.0)	**7 (0.7)**
Parainfluenza Virus 3	3 (1.2)	68 (12.8)	15 (5.8)	**86 (8.3)**
Respiratory Syncytial Virus A	17 (6.9)	85 (15.9)	17 (6.6)	**119 (11.4)**
Respiratory Syncytial Virus B	15 (6.1)	41 (7.7)	13 (5.0)	**69 (6.7)**
Adenovirus B/E	0 (0.0)	14 (2.6)	8 (3.1)	**22 (2.1)**
Adenovirus C	16 (6.5)	19 (3.6)	6 (2.3)	**41 (3.9)**

Table 21: Expected Value (Co-infections as Determined by eSensor RVP) Summary by Age Group in the Prospective Clinical Evaluation

Co-Infection	Age 0-1 (N = 270)	Age >1-5 (N = 136)	Age >5-21 (N = 127)	Age >21-65 (N = 333)	Age >65 (N = 171)	All Ages (N = 1037)
	N	N	N	N	N	N (%)
ADV B/E + Flu B	0	0	0	2	0	**2 (0.2)**
ADV B/E + HRV	0	2	0	0	0	**2 (0.2)**
ADV B/E + PIV3	3	0	0	0	0	**3 (0.3)**
ADV B/E + RSV A	1	1	0	0	0	**2 (0.2)**
ADV B/E + RSV B	0	1	0	0	0	**1 (0.1)**
ADV B/E + HMPV + HRV + RSV A + RSV B	1	0	0	0	0	**1 (0.1)**
ADV C + Flu B	1	0	0	0	0	**1 (0.1)**
ADV C + HMPV	3	0	0	0	0	**3 (0.3)**
ADV C + HRV	3	1	0	1	1	**6 (0.6**
ADV C + PIV3	0	0	0	1	0	**1 (0.1)**
ADV C + RSV A	2	2	0	0	0	**4 (0.4)**
ADV C + RSV B	1	0	0	1	1	**3 (0.3)**
ADV C + HRV + PIV3	1	0	0	0	0	**1 (0.1)**
ADV C + HRV + RSV A	1	0	0	0	0	**1 (0.1)**
Flu A + ADV B/E	0	0	1	0	0	**1 (0.1)**
Flu A + ADV C	1	1	0	2	2	**6 (0.6)**
Flu A + Flu B	0	0	1	1	0	**2 (0.2)**
Flu A + HMPV	0	0	0	1	1	**2 (0.2)**
Flu A + HRV	3	0	0	0	1	**4 (0.4)**
Flu A + PIV2	0	0	0	0	1	**1 (0.1)**
Flu A + PIV3	2	0	0	0	0	**2 (0.2)**

Co-Infection	Age 0-1 (N = 270)	Age >1-5 (N = 136)	Age >5-21 (N = 127)	Age >21-65 (N = 333)	Age >65 (N = 171)	All Ages (N = 1037)
	N	N	N	N	N	N (%)
Flu A + RSV A	1	0	0	0	0	1 (0.1)
Flu A + RSV B	0	1	0	1	0	2 (0.2)
Flu A + HRV + PIV3	2	0	0	0	0	2 (0.2)
Flu A + RSV A + RSV B	2	0	0	0	0	2 (0.2)
Flu A + ADV C + HRV + RSV A	1	0	0	0	0	1 (0.1)
Flu A + ADV C + HRV + PIV3	1	0	0	0	0	1 (0.1)
Flu B + HRV	1	0	1	1	1	4 (0.4)
Flu B + PIV3	0	2	0	0	1	3 (0.3)
Flu B + RSV A	2	0	2	0	1	5 (0.5)
Flu B + RSV B	0	1	0	0	0	1 (0.1)
Flu B + HRV + PIV2	0	1	0	0	0	1 (0.1)
Flu B + HRV + RSV A	2	0	0	0	0	2 (0.2)
HMPV + HRV	4	1	0	0	0	5 (0.5)
HMPV + PIV3	0	0	0	1	0	1 (0.1)
HMPV + RSV B	0	0	0	1	0	1 (0.1)
HRV + PIV1	2	0	0	0	0	2 (0.2)
HRV + PIV2	1	0	0	0	0	1 (0.1)
HRV + PIV3	9	0	1	1	0	11 (1.1)
HRV + RSV A	11	3	1	1	0	16 (1.6)
HRV + RSV B	6	2	0	0	0	8 (0.8)
HRV + PIV3 + RSV A	1	0	0	0	0	1 (0.1)
HRV + PIV3 + RSV B	0	1	0	0	0	1 (0.1)
PIV3 + RSV A	1	3	0	2	0	6 (0.6)
PIV3 + RSV B	0	0	0	1	0	1 (0.1)

Table 22: Expected Value (Co-infections as Determined by eSensor RVP) Summary by Site in the Prospective Clinical Evaluation

Co-Infection	Site 1 (N = 245)	Site 2 (N = 533)	Site 3 (N = 259)	All Sites (N = 1037)
	N	N	N	N (%)
ADV B/E + Flu B	0	0	2	2 (0.2)
ADV B/E + HRV	0	2	0	2 (0.2)
ADV B/E + PIV3	0	2	1	3 (0.3)
ADV B/E + RSV A	0	1	1	2 (0.2)
ADV B/E + RSV B	0	1	0	1 (0.1)
ADV B/E + HMPV + HRV + RSV A + RSV B	0	0	1	1 (0.1)
ADV C + Flu B	0	1	0	1 (0.1)
ADV C + HMPV	1	2	0	3 (0.3)

Co-Infection	Site 1 (N = 245)	Site 2 (N = 533)	Site 3 (N = 259)	All Sites (N = 1037)
	N	N	N	N (%)
ADV C + HRV	2	3	1	6 (0.6
ADV C + PIV3	0	1	0	1 (0.1)
ADV C + RSV A	1	3	0	4 (0.4)
ADV C + RSV B	3	0	0	3 (0.3)
ADV C + HRV + PIV3	0	1	0	1 (0.1)
ADV C + HRV + RSV A	0	1	0	1 (0.1)
Flu A + ADV B/E	0	1	0	1 (0.1)
Flu A + ADV C	3	2	1	6 (0.6)
Flu A + Flu B	0	1	1	2 (0.2)
Flu A + HMPV	1	0	1	2 (0.2)
Flu A + HRV	2	2	0	4 (0.4)
Flu A + PIV2	1	0	0	1 (0.1)
Flu A + PIV3	0	2	0	2 (0.2)
Flu A + RSV A	0	1	0	1 (0.1)
Flu A + RSV B	0	1	1	2 (0.2)
Flu A + HRV + PIV3	0	2	0	2 (0.2)
Flu A + RSV A + RSV B	0	2	0	2 (0.2)
Flu A + ADV C + HRV + RSV A	0	1	0	1 (0.1)
Flu A + ADV C + HRV + PIV3	0	1	0	1 (0.1)
Flu B + HRV	1	3	0	4 (0.4)
Flu B + PIV3	0	2	1	3 (0.3)
Flu B + RSV A	0	2	3	5 (0.5)
Flu B + RSV B	0	1	0	1 (0.1)
Flu B + HRV + PIV2	0	1	0	1 (0.1)
Flu B + HRV + RSV A	0	2	0	2 (0.2)
HMPV + HRV	2	2	1	5 (0.5)
HMPV + PIV3	0	0	1	1 (0.1)
HMPV + RSV B	1	0	0	1 (0.1)
HRV + PIV1	0	2	0	2 (0.2)
HRV + PIV2	0	1	0	1 (0.1)
HRV + PIV3	0	11	0	11 (1.1)
HRV + RSV A	3	12	1	16 (1.6)
HRV + RSV B	1	6	1	8 (0.8)
HRV + PIV3 + RSV A	0	1	0	1 (0.1)
HRV + PIV3 + RSV B	0	1	0	1 (0.1)
PIV3 + RSV A	2	4	0	6 (0.6)
PIV3 + RSV B	0	0	1	1 (0.1)

Prospective Clinical Study

All clinical specimens in the prospective clinical study were nasopharyngeal (NP) swab specimens, prospectively collected and tested during the 2010/11 influenza season at three North American clinical laboratories. Clinical laboratories were located in Cleveland, Ohio; Providence, RI; and Albuquerque, NM. Demographic details for patient population are summarized in Table 23. Study sites enrolled subjects from diverse demographic groups; about 40% of the specimens were obtained from patients enrolled at a hospital. The remaining specimens were collected from outpatients and patients in an emergency department. A total of 1182 patient samples were collected prospectively across the three clinical sites from January 2011 until May 2011. Out of these patient samples, 1037 were evaluable. A total of 145 samples were excluded for the following reasons: samples not tested within 5 days of specimen collection (72/145), operator and/or easyMAG mechanical errors (62/145), samples not retested (11/145). Out of the 1037 samples collected, an even split of patients were male and female. Approximately one quarter of the samples came from children under the age of 1, and patients aged 21-65 contributed the largest share of the samples.

Table 23: General Demographic Data for Prospectively Collected Specimens (N=1037)

Demographic	Site 1	Site 2	Site 3	All Sites
	N = 245 (%)	N = 533 (%)	N = 259 (%)	N = 1037 (%)
SEX				
Male	105 (42.9)	296 (55.5)	117 (45.2)	518 (50.0)
Female	140 (57.1)	237 (44.5)	142 (54.8)	519 (50.0)
AGE (yrs)				
0 – 1	46 (18.8)	197 (37.0)	27 (10.4)	270 (26.0)
> 1 – 5	20 (8.2)	94 (17.6)	22 (8.4)	136 (13.1)
> 5 – 21	19 (7.8)	82 (15.4)	26 (10.0)	127 (12.2)
> 21 – 65	97 (39.6)	106 (19.9)	130 (50.2)	333 (32.1)
> 65	63 (25.7)	54 (10.1)	54 (20.8)	171 (16.5)
SUBJECT STATUS				
Outpatient	7 (2.9)	219 (41.1)	90 (34.7)	316 (30.5)
Hospitalized	131 (53.5)	162 (30.4)	114 (44.0)	407 (39.2)
Emergency Department	107 (43.7)	152 (28.5)	55 (21.2)	314 (30.3)

A total of 1037 specimens were evaluated for all 14 RVP panel viruses with the prospectively collected samples, the performance for each respiratory virus was described by the clinical sensitivity and specificity. Sensitivity for a respiratory virus is the ability of the test to obtain positive results for this respiratory virus in the samples with positive results obtained by the comparator method for the particular virus. Specificity for a respiratory virus is the ability of the test to obtain negative results for this respiratory virus in the samples with negative results obtained by the comparator method for this respiratory virus. Depending on the comparator method used for a particular virus, performance is described as sensitivity/specificity or Positive Percent Agreement (PPA)/Negative Percent Agreement (NPA).

The performance of the RVP assay was compared to the established gold standard reference method of viral culture for most viral targets. For respiratory viruses in which culture was not available, a composite (multi-test) reference method (a predetermined algorithm that combined the results of a few tests) was used as the comparator method. As seen in Table 24, viral culture followed by DFA identification testing was used as the comparator method for Influenza A, Influenza B, RSV, Parainfluenza Viruses (PIV1, PIV2, PIV3), and adenovirus. Since viral culture cannot determine the subtype for influenza A, RSVs, and adenoviruses, these viruses were subtyped by an independently developed qRT-PCR assay or qPCR assay followed by bidirectional sequencing to determine the subtypes (Influenza A H1, Influenza A H3, Influenza A 2009 H1N1, RSVA, RSVB, ADVB/E and ADVC). HRV and HMPV were evaluated using two independently developed and validated qRT-PCR assays followed by bidirectional sequencing.

Table 24: Comparator Methods used to assess RVP performance

Virus (Analyte)	Comparator Method	Subtyping
Influenza A	Viral culture followed by DFA identification[1]	qRT-PCR + Bidirectional Sequencing
Influenza A H1		
Influenza A H3		
Influenza A 2009 H1N1		
RSV A		
RSV B		
Adenovirus B/E		
Adenovirus C		
Influenza B	Viral culture followed by DFA identification[2]	N/A
PIV 1		
PIV 2		
PIV 3		
Human Metapneumovirus	2 qRT-PCR (2 methods) with Bidirectional Sequencing[3]	N/A
Human Rhinovirus		

[1]Validated Performance of the eSensor RVP assay detecting Influenza A, RSV or ADV respectively was compared to viral culture followed by fluorescent antibody identification. "True" Influenza A, RSV or ADV positives respectively, were considered as any sample that tested positive for Influenza A, RSV or ADV respectively, by viral culture followed by DFA testing. True positive samples were subtyped using one analytically validated qRT-PCR assay with bi-directional sequence confirmation. The comparator assays were designed to amplify a different sequence from that amplified by the eSensor RVP assay(s). None of the comparator PCR assays overlapped any RVP amplicon sequence even if the same gene was targeted. "True" Influenza A H1, H3, or 2009 H1N1 positives, respectively, were considered as any sample that tested positive for Influenza A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched Influenza A/H1, A/H3, or A/2009 H1 sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values. "True" RSV A or RSV B positives, respectively, were considered as any sample that tested positive for Influenza A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched RSV A or RSV B sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values. "True" ADV C or ADV B/E positives, respectively, were considered as any sample that tested positive for Influenza A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched ADV C or ADV B/E sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values.

[2]Performance of the eSensor RVP assay detecting Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2 and Parainfluenza Virus 3 respectively was compared to viral culture followed by fluorescent antibody identification. "True" Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2 or Parainfluenza Virus 3 positives, respectively, were considered as any sample that tested positive for Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, or Parainfluenza Virus 3, respectively, by viral culture followed by DFA testing.

[3]Performance of the eSensor RVP assay detecting Human Rhinovirus or Human Metapneumovirus, respectively, was compared to a predetermined algorithm that used composite comparator methods. The methods consist of two analytically validated PCR assays followed by bi-directional sequencing. "True" Human

Rhinovirus or Human Metapneumovirus positives, respectively, were considered as any sample that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched Human Rhinovirus or Human Metapneumovirus sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih. gov), respectively, with acceptable E-values.

Sensitivity or positive percent agreement (PPA) was calculated by dividing the number of true positive (TP) results by the sum of true positive and false negative (FN) results, while specificity or negative percent agreement (NPV) was calculated by dividing the number of true negative (TN) results by the sum of true negative and false positive (FP) results. A TP result was one where the positive RVP result matched the positive reference/comparator result, while a TN result was one whereby a negative RVP result matched a negative reference/comparator result. The two-sided 95% confidence interval was also calculated. The results are summarized below.

Table 25: Performance in Prospective Clinical Specimens (N=1037)

Virus (Analyte)	Sensitivity			Specificity		
	TP/(TP+FN)	Percent	95% CI	TN/(TN+FP)	Percent	95% CI
Influenza A[1]	132/137[a]	96.4%	91.7% - 98.8%	850/897[b]	94.8%	93.1% - 96.1%
Influenza A H1*	0/0	NA	NA	1027/1027	100.0%	99.6% - 100.0%
Influenza A H3	74/74	100.0%	95.1% - 100.0%	927/952[c]	97.4%	96.2% - 98.3%
Influenza A 2009 H1N1	49/49	100.0%	92.7% - 100.0%	956/971[d]	98.5%	97.5% - 99.1%
Influenza B	64/69[e]	92.8%	83.9% - 97.6%	947/965[f]	98.1%	97.1% - 98.9%
Parainfluenza Virus 1*	4/4	100.0%	39.8% - 100.0%	1029/1030[j]	99.9%	99.5% - 100.0%
Parainfluenza Virus 2*	5/6[i]	83.3%	35.9% - 99.6%	1026/1028[k]	99.8%	99.3% - 100.0%
Parainfluenza Virus 3	64/68[l]	94.1%	85.6% - 98.4%	944/966[m]	97.7%	96.6% - 98.6%
Respiratory Syncytial Virus A	68/68	100.0%	94.7% - 100.0%	905/956[n]	94.7%	93.1% - 96.0%
Respiratory Syncytial Virus B	28/28	100.0%	87.7% - 100.0%	955/996[o]	95.9%	94.5% - 97.0%
Adenovirus B/E*	13/13	100.0%	75.3% - 100.0%	1012/1021[p]	99.1%	98.3% - 99.5%
Adenovirus C*	6/6	100.0%	54.1% - 100.0%	993/1028[q]	96.6%	95.3% - 97.5%
Virus (Analyte)	**PPA**			**NPA**		
	TP/(TP+FN)	**Percent**	**95% CI**	**TN/(TN+FP)**	**Percent**	**95% CI**
Human Metapneumovirus	55/55	100.0%	93.5% - 100.0%	979/981[g]	99.8%	99.3% - 100.0%
Human Rhinovirus	132/148	89.2%	83.0% - 93.7%	853/888[h]	96.1%	94.6% - 97.3%

*These viral targets were supplemented with retrospective samples as shown below.

[1]Influenza A results contain 14 Flu A samples without a positive subtype and 123 samples with either Influenza A H3 or 2009 H1N1 positive results.

[a] Flu A was not detected in all 5 RVP False Negative samples using independently developed and validated qPCR assays.

[b] Flu A viruses were confirmed positive in 35/47 RVP False Positive samples using bidirectional sequencing.

[c] Flu A H3 viruses were confirmed positive in 22/25 RVP False Positive samples using bidirectional sequencing.

[d] Flu A 2009 H1N1 viruses were confirmed positive in 14/15 RVP False Positive samples using bidirectional sequencing.

[e] Flu B was not detected in 4/5 RVP False Negative samples using bidirectional sequencing.

[f] Flu B was confirmed positive in 11/18 RVP False Positive samples using bidirectional sequencing.

[g] hMPV was confirmed positive in 1/2 RVP False Positive samples using bidirectional sequencing.

[h] HRV was confirmed positive in 7/35 RVP False Positive samples using bidirectional sequencing.

[i] PIV 1 was not detected in this RVP False Positive sample by bidirectional sequencing.

[j] PIV 2 was not detected in this RVP False Negative sample using independently developed and validated qPCR assays.

[k] PIV 2 virus was confirmed positive in 0/2 RVP False Positive samples by bidirectional sequencing.

[l] PIV 3 was not detected in 4/4 RVP False Negative samples using independently developed and validated qPCR assays.

[m] PIV 3 virus was confirmed positive in 10/22 RVP False Positive samples using bidirectional sequencing.

[n] RSV A were confirmed positive in 43/51 RVP False Positive samples using bidirectional sequencing.

[o] RSV B was confirmed positive in 35/41 RVP False Positive samples using bidirectional sequencing.

[p] ADV B/E was confirmed positive in 8/9 RVP False Positive samples using bidirectional sequencing.

[q] ADV C was confirmed positive in 16/35 False Positive samples using bidirectional sequencing.

The eSensor RVP system detected a total of 128 mixed infections in the prospective clinical evaluation (1037 tested and analyzed specimens). This represents 18.4% of the total positive specimens (128/696). One hundred fourteen (114/128; 89.1%) were double infections, eleven (11/128; 8.6%) were triple infections, and three (3/128; 2.3%) samples with four or more RVP analytes were identified. Ninety five of the 128 samples contained one or more analytes that the reference/comparator method failed to detect.

Table 26: Distinct Co-infection Combinations Detected by the eSensor RVP Assay in the Prospective Clinical Trial

Distinct Co-infection Combinations Detected by eSensor RVP					Total Number of Co-infections	Number of Discrepant Co-infections	Discrepant Analyte(s)
Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5			
ADV B/E	Flu B				2	2	ADV B (2), Flu B (1)
ADV B/E	HRV				2	0	
ADV B/E	PIV3				3	3	ADV B (3)
ADV B/E	RSV A				2	2	ADV B (1), RSV A (2)
ADV B/E	RSV B				1	1	RSV B (1)
ADV B/E	HMPV	HRV	RSV A	RSV B	1	1	RSV A (1), RSV B (1)
ADV C	Flu B				1	1	ADV C (1)
ADV C	HMPV				3	3	ADV C (3)
ADV C	HRV				6	4	ADV C (4), HRV (1)
ADV C	PIV3				1	1	ADV C (1)
ADV C	RSV A				4	4	ADV C (3), RSV A (2)[r]
ADV C	RSV B				3	3	ADV C (3), RSV B (2)
ADV C	HRV	PIV3			1	1	ADV C (1)
ADV C	HRV	RSV A			1	0	
Flu A	ADV B/E				1	1	Flu A
Flu A	ADV C				6	6	ADV C (6)
Flu A	Flu B				2	2	Flu A (2), HRV (1)
Flu A	HMPV				2	2	H1N1 (1), H3 (1), HMPV (1)
Flu A	HRV				4	2	H1N1 (1), HRV (2)
Flu A	PIV2				1	1	PIV2 (1)
Flu A	PIV3				2	2	Flu A (1), PIV3 (2)
Flu A	RSV A				1	1	RSV A (1)

Distinct Co-infection Combinations Detected by eSensor RVP					Total Number of Co-infections	Number of Discrepant Co-infections	Discrepant Analyte(s)*
Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5			
Flu A	RSV B				2	2	RSV B (2)
Flu A	HRV	PIV3			2	1	H1N1 (1)
Flu A	RSV A	RSV B			2	2	RSV A (2), RSV B (2)
Flu A	ADV C	HRV	RSV A		1	1	ADV C (1), HRV (1)
Flu A	ADV C	HRV	PIV3		1	1	ADV C (1), Flu A (1), PIV3 (1)
Flu B	HRV				4	2	Flu B (1), HRV (1)
Flu B	PIV3				3	3	Flu B (2), PIV3 (2)
Flu B	RSV A				5	5	Flu B (2), RSV A (5)
Flu B	RSV B				1	1	RSV B (1)
Flu B	HRV	PIV2			1	1	HRV (1), PIV2 (1)
Flu B	HRV	RSV A			2	1	RSV A (1)
HMPV	HRV				5	1	HMPV (1)
HMPV	PIV3				1	0	
HMPV	RSV B				1	1	RSV B (1)
HRV	PIV1				2	1	PIV1 (1)
HRV	PIV2				1	1	HRV (1)
HRV	PIV3				11	4	HRV (4), PIV3 (2)
HRV	RSV A				16	9	HRV (5), RSV A (6)
HRV	RSV B				8	6	HRV (1), RSV B (5)
HRV	PIV3	RSV A			1	1	RSV A (1)
HRV	PIV3	RSV B			1	1	RSV B (1)
PIV3	RSV A				6	6	PIV3 (4), RSV A (5)
PIV3	RSV B				1	1	PIV3 (1), RSV B (1)
Total Number of Co-infections					128	95	117/278[1]
Total Number of Double Infections					114	85	99/232
Total Number of Triple Infections					11	8	11/33
Total Number of Quadruple Infections					2	2	5/8
Total Number of Quintuple Infections					1	1	2/5

*A discrepant co-infection or discrepant analyte was defined as one that was detected by RVP but not the reference/comparator methods.

[1]117/117 discrepant analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 58/117 cases.

[a]6/6 discrepant ADV B/E analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 5/6 cases

[b]24/24 discrepant ADV C analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 11/24 cases

[c]6/6 discrepant Flu B analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 3/6 cases

[d]4/4 discrepant Flu A 2009 H1N1 analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 4/4 cases

[e]1/1 discrepant Flu A H3 analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 1/1 cases

[f]2/2 discrepant HMPV analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 1/2 cases

[g]19/19 discrepant HRV analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 3/19 cases

[h]12/12 discrepant PIV3 analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 3/12 cases

[i]27/27 discrepant RSV A analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 17/27 cases

[j]17/17 discrepant RSV B analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 11/17 cases

Table 27: Additional Co-Infection Combinations Detected by Reference/Comparator Methods, But Not by the eSensor RVP Assay in the Prospective Clinical Trial

Distinct Co-Infection Combinations*		Total Number of Co-Infections	Number of Discrepant Co-infections	Discrepant Analyte(s)
Analyte 1	Analyte 2			
Flu B	HRV	6	3	Flu B (2), HRV (3)
Flu B	RSV B	1	1	Flu B (1), RSV B (1)
HRV	PIV3	13	3	HRV (3), PIV3 (3)

*This table includes only co-infections that were detected by the reference/comparator method but not by RVP; the remaining co-infections detected by the reference/comparator method are already represented in Table above.

Table 28: Mixed Infections Detected by eSensor RVP in Prospective Samples

Organism Combinations	Number of Samples	% of Samples Analyzed (N=1037)	Organism Combinations	Number of Samples	% of Samples Analyzed (N=1037)
ADV B/E + Flu B	2	0.2	Flu A + HRV + PIV3	2	0.2
ADV B/E + HRV	2	0.2	Flu A + RSV A + RSV B	2	0.2
ADV B/E + PIV3	3	0.3	Flu A + ADV C + HRV + PIV3	1	0.1
ADV B/E + RSV A	2	0.2	Flu A + ADV C + HRV + RSV A	1	0.1
ADV B/E + RSV B	1	0.1	Flu B + HRV	4	0.4
ADV B/E + HMPV + HRV + RSV A + RSV B	1	0.1	Flu B + PIV3	3	0.3
ADV C + Flu B	1	0.1	Flu B + RSV A	5	0.5
ADV C + HMPV	3	0.3	Flu B + RSV B	1	0.1
ADV C + HRV	6	0.6	Flu B + HRV + PIV2	1	0.1
ADV C + PIV3	1	0.1	Flu B + HRV + RSV A	2	0.2
ADV C + RSV A	4	0.4	HMPV + HRV	5	0.5
ADV C + RSV B	3	0.3	HMPV + PIV3	1	0.1
ADV C + HRV + PIV3	1	0.1	HMPV + RSV B	1	0.1
ADV C + HRV + RSV A	1	0.1	HRV + PIV1	2	0.2

Organism Combinations	Number of Samples	% of Samples Analyzed (N=1037)	Organism Combinations	Number of Samples	% of Samples Analyzed (N=1037)
Flu A + ADV B/E	1	0.1	HRV + PIV2	1	0.1
Flu A + ADV C	6	0.6	HRV + PIV3	11	1.1
Flu A + Flu B	2	0.2	HRV + RSV A	16	1.6
Flu A + HMPV	2	0.2	HRV + RSV B	8	0.8
Flu A + HRV	4	0.4	HRV + PIV3 + RSV A	1	0.1
Flu A + PIV2	1	0.1	HRV + PIV3 + RSV B	1	0.1
Flu A + PIV3	2	0.2	PIV3 + RSV A	6	0.6
Flu A + RSV A	1	0.1	PIV3 + RSV B	1	0.1
Flu A + RSV B	2	0.2	Total Mixed Infections	128	12.3

93% (963/1037) of the evaluable prospective clinical specimens yielded valid results on the first attempt. Invalid results or no results were obtained for the remaining 74 specimens (45 of which generated results on the first run, but required retesting due to a negative control failure caused by operator error). Data generated from the retests was used in the final analysis. All 74 specimens yielded valid results after a single retest when tested according the retest recommendations.

Testing of Preselected Archived Samples

Banked samples previously characterized as positive for Influenza A H1, Parainfluenza Virus 1, Parainfluenza Virus 2, Adenovirus B/E, and Adenovirus C were used to supplement the performance studies for these analytes. These frozen banked samples were collected from various sites across the United States or from the Centers for Disease Control and Prevention (CDC). Upon arrival at GenMark, banked samples were blinded and intermixed with negative samples before being sent for testing, which was conducted by multiple sites involved in the prospective analysis of the patient samples. Testing of the banked samples was performed identically to prospectively-collected patient specimens. Results from the banked samples are presented separately from the prospectively collected specimens.

A total of 343 retrospective banked samples were collected for analysis. Out of this sample set, 11 samples were sent which didn't contain a banked viral target so these eleven samples were not tested further. Eight additional samples were excluded as they didn't contain a banked viral target as originally reported by the collection site and confirmed by comparator testing. Two samples reported errors on targets but were not retested as indicated. One sample was not sequenced. One sample had an internal control failure but was not retested as indicated. After these data were excluded, a total of 320 banked samples (including negative samples) for 5 viral targets were collected and analyzed.

With the exception of Flu A H1 samples, these banked samples were also sent to Beckman Coulter for comparator testing, and the results from the Beckman Coulter testing were compared to the results obtained by the eSensor RVP. Since the Flu A H1 samples came from the Centers for Disease Control and Prevention and were verified to be Flu A H1, these samples were not sent to Beckman Coulter for further testing. The results are summarized in Table 29.

Table 29: Performance in Retrospective Clinical Specimens (N=320)

Virus	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP+FN)	Percent	95% CI	TN/(TN+FP)	Percent	95% CI
Influenza A H1	29/30	96.7%	82.8% - 99.9%	290/290	100%	98.7% - 100.0%
Parainfluenza Virus 1	25/25	100.0%	86.3% - 100.0%	289/295	98.0%	95.6% - 99.3%
Parainfluenza Virus 2	26/26	100.0%	86.8% - 100.0%	284/294	96.6%	93.8% - 98.4%
Adenovirus B/E	25/25	100.0%	86.3% - 100.0%	290/295	98.3%	96.1% - 99.4%
Adenovirus C	16/16	100.0%	80.6% - 100.0%	270/304	88.8%	84.8% - 91.9%

eSensor RVP Performance in Fresh vs. Frozen Clinical Specimen

Simulated viral specimens were prepared by spiking viral transport media (Remel M5) with two different concentrations of ADV C viral culture (3x LoD and 1x LoD). To evaluate the performance of frozen specimens, 128 aliquots of ADV C (64 replicates each at 3x and 1x LoD) were prepared. Sixty four aliquots (32 at each testing concentration) were tested immediately after preparation (fresh) while 64 aliquots were tested after undergoing two freeze/thaw cycles (frozen). Positive percent agreement between RVP results from fresh versus frozen aliquots for all concentrations tested was calculated. The positive percent agreement between RVP results from fresh versus frozen aliquots was 100% (95% confidence interval 89.3% - 100%).

Prospective 2X2 Performance Tables:

Table 30: Prospective Influenza A Results

Influenza A			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	132	47[a]	179
Negative	5[b]	850	855
Total	137	897	1034
Sensitivity: 96.4% (95% CI: 91.7% - 98.8%)			
Specificity: 94.8% (95% CI: 93.1% - 96.1%)			

a Influenza A virus was confirmed positive in 35/47 RVP False Positive samples using bidirectional sequencing.
b Influenza A virus was not detected in all 5 RVP False Negative samples using independently developed and validated qPCR assays.

Table 31: Prospective Influenza A H1 Results

Influenza A H1			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	0	0	0
Negative	0	1027	1027
Total	0	1027	1027
Sensitivity: N/A			
Specificity: 100.0% (95% CI: 99.6% - 100.0%)			

Table 32: Prospective Influenza A H3 Results

Influenza A H3			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	74	25[a]	99
Negative	0	927	927
Total	74	952	1026
Sensitivity: 100.0% (95% CI: 95.1% - 100.0%)			
Specificity: 97.4% (95% CI: 96.2% - 98.3%)			

a Influenza A H3 virus was confirmed positive in 22/25 RVP False Positive samples using bidirectional sequencing.

Table 33: Prospective Influenza A 2009 H1N1 Results

Influenza A 2009 H1N1			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	49	15[a]	64
Negative	0	956	956
Total	49	971	1020
Sensitivity: 100.0% (95% CI: 92.7% - 100.0%)			
Specificity: 98.5% (95% CI: 97.5% - 99.1%)			

a Influenza A 2009 H1N1 virus was confirmed positive in 14/15 RVP False Positive samples using bidirectional sequencing.

Table 34: Prospective Influenza B Results

Influenza B			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	64	18[a]	82
Negative	5[b]	947	952
Total	69	965	1034
Sensitivity: 92.8% (95% CI: 83.9% - 97.6%)			
Specificity: 98.1% (95% CI: 97.1% - 98.9%)			

a Influenza B virus was confirmed positive in 11/18 RVP False Positive samples using bidirectional sequencing.

b Influenza B virus was not detected in 4/5 RVP False Negative samples using independently developed and validated qPCR assays.

Table 35: Prospective RSVA Results

Respiratory Syncytial Virus A			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	68	51[a]	119
Negative	0	905	905
Total	68	956	1024
Sensitivity: 100.0% (95% CI: 94.7% - 100.0%)			
Specificity: 94.7% (95% CI: 93.1% - 96.0%)			

a Respiratory Syncytial Virus type A was confirmed positive in 43/51 RVP False Positive samples using bidirectional sequencing.

Table 36: Prospective RSVB Results

Respiratory Syncytial Virus B			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	28	41[a]	69
Negative	0	955	955
Total	28	996	1024
Sensitivity: 100.0% (95% CI: 87.7% - 100.0%)			
Specificity: 95.9% (95% CI: 94.5% - 97.0%)			

a Respiratory Syncytial Virus type B was confirmed positive in 35/41 RVP False Positive samples using bidirectional sequencing.

Table 37: Prospective PIV1 Results

Parainfluenza Virus 1			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	4	1[a]	5
Negative	0	1029	1029
Total	4	1030	1034
Sensitivity: 100.0% (95% CI: 39.8% - 100.0%)			
Specificity: 99.9% (95% CI: 99.5% - 100.0%)			

a PIV 1 was not detected in this RVP False Positive sample by bidirectional sequencing.

Table 38: Prospective PIV2 Results

Parainfluenza Virus 2			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	5	2[a]	7
Negative	1[b]	1026	1027
Total	6	1028	1034
Sensitivity: 83.3% (95% CI: 35.9% - 99.6%)			
Specificity: 99.8% (95% CI: 99.3% - 100.0%)			

a Parainfluenza type 2 virus was confirmed positive in 0/2 RVP False Positive samples by bidirectional sequencing.
b Parainfluenza type 2 virus was not detected in this RVP False Negative sample using independently developed and validated qPCR assays.

Table 39: Prospective PIV3 Results

Parainfluenza Virus 3			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	64	22[a]	86
Negative	4[b]	944	948
Total	68	966	1034
Sensitivity: 94.1% (95% CI: 85.6% - 98.4%)			
Specificity: 97.7% (95% CI: 96.6% - 98.6%)			

a Parainfluenza type 3 virus was confirmed positive in 10/22 RVP False Positive samples using bidirectional sequencing.
b Parainfluenza type 3 virus was not detected in 4/4 RVP False Negative samples using independently developed and validated qPCR assays.

Table 40: Prospective HMPV Results

Human Metapneumovirus			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	55	2[a]	57
Negative	0	979	979
Total	55	981	1036
Sensitivity: 100.0% (95% CI: 93.5% - 100.0%)			
Specificity: 99.8% (95% CI: 99.3% - 100.0%)			

a Human metapneumovirus was confirmed positive in 1/2 RVP False Positive samples using bidirectional sequencing.

Table 41: Prospective HRV Results

Human Rhinovirus			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	132	35[a]	167
Negative	16	853	869
Total	148	888	1036
Sensitivity: 89.2% (95% CI: 83.0% - 93.7%)			
Specificity: 96.1% (95% CI: 94.6% - 97.3%)			

a Human rhinovirus was confirmed positive in 7/35 RVP False Positive samples using bidirectional sequencing.

Table 42: Prospective ADV B/E Results

Adenovirus B/E			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	13	9[a]	22
Negative	0	1012	1012
Total	13	1021	1034
Sensitivity: 100.0% (95% CI: 75.3% - 100.0%)			
Specificity: 99.1% (95% CI: 98.3% - 99.5%)			

a Adenovirus type B/E was confirmed positive in 8/9 RVP False Positive samples using bidirectional sequencing.

Table 43: Prospective ADV C Results

Adenovirus C			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	6	35[a]	41
Negative	0	993	993
Total	6	1028	1034
Sensitivity: 100.0% (95% CI: 54.1% - 100.0%)			
Specificity: 96.6% (95% CI: 95.3% - 97.5%)			

a Adenovirus type C was confirmed positive in 16/35 RVP False Positive samples using bidirectional sequencing.

Retrospective 2X2 Performance Tables:

Table 44: Retrospective Influenza A H1 Results

Influenza A (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	29	0	29
Negative	1	290	291
Total	30	290	320
Positive Percent Agreement: 96.7% (95% CI: 82.8% - 99.9%)			
Negative Percent Agreement: 100.0% (95% CI: 98.7% - 100.0%)			

Table 45: Retrospective PIV1 Results

PIV1 (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	25	6	31
Negative	0	289	289
Total	25	295	320
Positive Percent Agreement: 100.0% (95% CI: 86.3% - 100.0%)			
Negative Percent Agreement: 98.0% (95% CI: 95.6% - 99.3%)			

Table 46: Retrospective PIV2 Samples

PIV2 (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	26	10	36
Negative	0	284	284
Total	26	294	320
Positive Percent Agreement: 100.0% (95% CI: 86.8% - 100.0%)			
Negative Percent Agreement: 96.6% (95% CI: 93.8% - 98.4%)			

Table 47: Retrospective ADV B/E Results

ADV B/E (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	25	5	30
Negative	0	290	290
Total	25	295	320
Positive Percent Agreement: 100.0% (95% CI: 86.3% - 100.0%)			
Negative Percent Agreement: 98.3% (95% CI: 96.1% - 99.4%)			

Table 48: Retrospective ADV C Results

ADV C (Banked Samples)			
eSensor RVP	**Reference**		
	Positive	**Negative**	**Total**
Positive	16	34	50
Negative	0	270	270
Total	16	304	320
Positive Percent Agreement: 100.0% (95% CI: 80.6% - 100.0%)			
Negative Percent Agreement: 88.8% (95% CI: 84.8% - 91.9%)			

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

SEP 10 2012

GenMark Diagnostics, Inc.
c/o Joel Centeno
VP Regulatory, Quality, Clinical
5964 La Place Court
Carlsbad, CA 92008

Re: k113731
Trade/Device Name: eSensor® Respitory Viral Panel (RVP)
Regulation Number: 21 CFR 866.3980
Regulation Name: Respitory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OCC, OEM, OOU, OEP, OQW, NSU, OUL, JJH
Dated: September 4, 2012
Received: September 6, 2012

Dear Mr. Centeno:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice

requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use Form

510(k) Number (if known): K113731

Device Name: eSensor® Respiratory Viral Panel (RVP)

Indications for Use:

The eSensor® Respiratory Viral Panel (RVP) is a qualitative nucleic acid multiplex in vitro diagnostic test intended for use on the eSensor XT-8TM system for the simultaneous detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory infection.

The following virus types and subtypes are identified using the eSensor RVP: Influenza A, Influenza A H1 Seasonal Subtype, Influenza A H3 Seasonal Subtype, Influenza A 2009 H1N1 subtype, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Human Metapneumovirus, Human Rhinovirus, Adenovirus species B/E, and Adenovirus species C.

The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and epidemiological information.

Negative results do not preclude respiratory viral infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive results do not rule out bacterial infection, or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence and radiography) and clinical presentation must be taken into consideration in the final diagnosis of respiratory viral infection.

Performance characteristics for Influenza A were established during the 2010/2011 influenza season when Influenza A 2009 H1N1 and H3N2 were the predominant Influenza A viruses in circulation. When other Influenza A viruses emerge, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Prescription Use X (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Tamara Feldblyum

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K113731